Title: Associations between the vaginal microbiome and *Candida* colonization in women of reproductive age

Authors and affiliations
Mr. Brett A. TORTELLI B.A.[1,2], Warren G. LEWIS Ph.D.[2], Jenifer E. ALLSWORTH Ph.D.[6], Mr. Nadum MEMBER-MENEH B.S.[2,3], Ms. Lynne R. FOSTER B.S.[2,3], Hilary E. RENO M.D. Ph.D.[4], Jeffrey F. PEIPERT M.D., Ph.D. [7], Justin C. FAY* Ph.D.[1,8], Amanda L. LEWIS* Ph.D.[2,3,5]

Author affiliations:
[1]Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA.
[2]Center for Women's Infectious Disease Research, Washington University School of Medicine, St. Louis, MO, USA.
[3]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA.
[4]Division of Infectious Diseases, Washington University School of Medicine, St. Louis, MO 63110, USA.
[5]Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO 63110, USA.
[6]Department of Biomedical and Health Informatics, University of Missouri-Kansas City School of Medicine, Kansas City, MO, USA.
[7]Department of Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN, USA.
[8]Department of Biology, University of Rochester, Rochester, NY, USA.

Conflicts of interest: Dr. Peipert serves on advisory boards for Bayer and CooperSurgical, and has received research support from Merck, Bayer, and CooperSurgical/Teva. Although not directly related to the work, A. L. L. received personal (consulting) fees from companies involved in making diagnostics or treatments for bacterial vaginosis (Talis Biomedical Corporation, Tennor Therapeutics, and Toltec Pharmaceuticals) and both Dr. AL and WG Lewis have performed research sponsored by Metis Therapeutics and Metrodora Therapeutics. Remaining authors report no conflict of interest.

Source of funding: Funding for the Contraceptive CHOICE Project was provided by an anonymous foundation. This study was supported in part by a March of Dimes grant (Prematurity Research Center) and a pilot grant from the same (ALL). Funding was provided by a pilot grant from the Center for Women's Infectious Disease Research (JCF) and by the NIH: R01 AI114635 (ALL and WGL), R01 AI127554 (WGL), and by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the NIH: F30HD094435 (BAT). The sponsors had no role in the study design, the collection, analysis or interpretation of data, or the decision to submit the article for publication. The content of this study is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
Paper presentation information: This manuscript was presented in part at the 14th ASM Conference on *Candida* and Candidiasis in Providence RI, April 15-19th 2018 and the American Physician Scientist Association 15th Annual Meeting in Chicago IL, April 5-7th 2019.

Amanda L. Lewis, Ph.D.
Washington University School of Medicine
Department of Molecular Microbiology
660 South Euclid Ave
Saint Louis, MO, 63119
Office phone: 314-286-0016
E-mail: allewis@wustl.edu

Justin C. Fay, Ph.D.
University of Rochester
Department of Biology
319 Hutchison Hall
Rochester, NY 14627-0211
Office phone: 585-275-2998
E-mail: justin.fay@rochester.edu

Word Count
Abstract: 471
Main Text: 3000

Condensation: *Lactobacillus iners*-dominant vaginal microbiomes are more likely to harbor vaginal *Candida* than *Lactobacillus crispatus*-dominant vaginal microbiomes.

Short Title: *Candida* and the vaginal microbiome

AJOG at a Glance:

A. The purpose of the study was to characterize the relationship between the composition of the vaginal microbiome and *Candida* colonization among non-pregnant women.
B. Women with *Lactobacillus iners*-dominant microbiomes were more likely to harbor *Candida* than women with *Lactobacillus crispatus*-dominant microbiomes. *In vitro* data suggests higher production of lactic acid by *Lactobacillus crispatus* compared to *Lactobacillus iners* may contribute to differential anti-*Candida* activity. Neutralization of pH eliminated the anti-*Candida* activity secreted by lactobacilli.
C. Consideration of *Candida* as part of the vaginal microbiome may have utility for understanding different relationships between vaginal microbiome and adverse outcomes.

Word Count
Abstract: 471
Main Text: 3000

Background: The composition of bacteria within the vaginal microbiome has garnered a lot of recent attention and has been associated with reproductive health and disease. Despite the common occurrence of yeast (primarily *Candida*) within the vaginal microbiome, there is still an incomplete picture of relationships between yeast and bacteria (especially lactobacilli), as well as how such associations are governed. Such relationships could be important to a more holistic understanding of the vaginal microbiome and its connection to reproductive health.

Objective: To perform molecular characterization of clinical specimens to define associations between vaginal bacteria (especially *Lactobacillus* species) and *Candida* colonization. *In vitro* studies were conducted to test the two most common dominant *Lactobacillus* species (*Lactobacillus crispatus* and *Lactobacillus iners*) in their ability to inhibit *Candida* growth and to examine the basis for such inhibition.

Study Design: A nested cross-sectional study of reproductive age women from the Contraceptive CHOICE Project was conducted. Vaginal swabs from 299 women were selected to balance race and BV status, resulting in similar representation of black and white women in each of the three Nugent score categories [normal (0-3), intermediate (4-6), and bacterial vaginosis (7-10)]. Sequencing of the 16S ribosomal gene (V4 region) was used to determine the dominant *Lactobacillus* species present (primarily *L. iners* and *L. crispatus*), defined as >50% of the community. Subjects without dominance by a single *Lactobacillus* species were classified as Diverse. A *Candida*-specific qPCR targeting the internally transcribed spacer 1 (ITS1) was validated using vaginal samples collected from a second cohort of women and used to assess *Candida* colonization. 255 nonpregnant women with sufficient bacterial biomass for analysis were included in the final analysis. Generalized linear models were employed to evaluate associations between *Lactobacillus* dominance, sociodemographic and risk characteristics and

Word Count
Abstract: 471
Main Text: 3000

vaginal *Candida* colonization. In separate *in vitro* studies, the potential of cell-free supernatants from *L. crispatus* and *L. iners* cultures to inhibit *Candida* growth was evaluated.

Results: Forty-two women (16%) were vaginally colonized with *Candida*. Microbiomes characterized as Diverse (38%), *L. iners*-dominant (39%), and *L. crispatus*-dominant (20%) were the most common. The microbiome, race and *Candida* colonization co-varied with a higher prevalence of *Candida* among black women and *L. iners*-dominant communities compared to white women and *L. crispatus*-dominant communities. *L. iners*-dominant communities were more likely to harbor *Candida* than *L. crispatus*-dominant communities (OR = 2.85, 95% CI: 1.03 to 7.21; Fisher's Exact, p = 0.048). *In vitro*, *L. crispatus* produced greater concentrations of lactic acid and exhibited significantly more pH-dependent growth inhibition of *C. albicans*, suggesting a potential mechanism for the clinical observations.

Conclusion: In nonpregnant women, *L. iners*-dominant communities were significantly more likely to harbor *Candida* than *L. crispatus*-dominant communities, suggesting that *Lactobacillus* species have different relationships with *Candida*. *In vitro* experiments indicate that *L. crispatus* may impede *Candida* colonization more effectively than *L. iners* through a greater production of lactic acid.

Key Words: vaginal microbiome, *Candida*, *Lactobacillus*, race, pH

Word Count
Abstract: 471
Main Text: 3000

Introduction

The human vagina is a dynamic ecosystem that hosts microbes from diverse taxa. Profiling 16S ribosomal gene diversity has expanded our understanding of the vaginal microbiome, allowing exploration of links between bacterial composition and reproductive outcomes. Vaginal microbial communities can be clustered into five common community types.[1] Four of these are dominated by a single *Lactobacillus* species: *L. crispatus*, *L. gasseri*, *L. iners*, or *L. jensenii*. The final community type (often described as "Diverse") has few lactobacilli and exhibits greater representation of anaerobic bacteria such as *Gardnerella vaginalis, Atopobium vaginae* and *Prevotella spp.*[1] The prevalence of these community types varies with race and ethnicity; black and Hispanic women more frequently host *L. iners*-dominant and Diverse communities than white women, who more frequently host *L. crispatus*-dominant communities.[1,2] Diverse communities often harbor bacterial taxa that are abundant during bacterial vaginosis (BV), a condition diagnosed by clinical (Amsel) criteria or by Nugent scoring,[3] a 0-10 scale generated by scoring bacterial morphotypes in Gram-stained vaginal smears (0-3, normal; 4-6, intermediate; 7-10, BV). BV is associated with increased risks of sexually transmitted infections and adverse reproductive outcomes.[4]

Candida (most commonly *C. albicans*) is a common member of the vaginal microbiome (found in ~30% of women[5]). The prevalence of non-*albicans* species among women with vaginal *Candida* varies, ranging from ~10-30%.[5-9] Vaginal *Candida* colonization may lead to vulvovaginal candidiasis (VVC), characterized by an aggressive host response to *Candida* overgrowth.[10] However, *Candida* colonization is frequently asymptomatic and not all women

Word Count
Abstract: 471
Main Text: 3000

colonized with *Candida* go on to experience VVC.[5] Vaginal *Candida* colonization has also been linked to other adverse reproductive outcomes.[8,11-16]

Several prior studies have examined relationships between vaginal bacteria and *Candida*. A few of these studies implicate an abundance of lactobacilli with a greater likelihood of harboring *Candida*.[5,6,17] Other studies suggest there may be co-occurrence of *Candida* with some BV-associated bacteria,[18-21] and specifically that *Candida* may be correlated with the simultaneous presence of both lactobacilli and BV-associated bacteria.[19-21] An important limitation is that prior studies, whether using molecular or culture-based techniques, have not distinguished between lactobacilli at the species level. This is a significant limitation, which if resolved, may shed light on why some women are so prone to *Candida* colonization and candidiasis.

Taken together with the prior studies above, several considerations led us to hypothesize that *L. iners* in particular may support the co-occurrence of *Candida*, *especially compared to L. crispatus*. *L. iners* is unique among the lactobacilli in being prevalent within less stable Nugent intermediate and BV communities[1,22,23] and in producing a cytolytic toxin.[24,25] Furthermore, *L. iners* dominance has been associated with other negative health outcomes such as increased risks of *Chlamydia trachomatis* infection,[26] incident BV,[27] defects in vaginal mucus that compromise antiviral barrier function,[28] and cytokine signatures linked with HIV risk.[29] We performed two types of studies to test our hypothesis that *L. iners* may preferentially support *Candida* colonization 1) a molecular evaluation of clinical specimens, and 2) *in vitro* growth inhibition studies.

Word Count
Abstract: 471
Main Text: 3000

Methods

Study design:

This nested cross-sectional study uses samples and questionnaire data collected by the Contraceptive CHOICE Project (CHOICE)[30] according to Washington University IRB-approved protocol 201108155. In total, 9256 women from the St. Louis-area gave informed consent from August 2007 through September 2011. For this nested study, 299 women enrolled from 08/2008-06/2009 were selected based on power calculations made from preliminary data. Women enrolled in the CHOICE study were between the ages of 14 and 45, reported sexual activity in the past six months or anticipated sexual activity with a male partner and were seeking contraception. Women with a history of tubal ligation or hysterectomy were excluded. All women underwent a pregnancy test. Vaginal swab specimens were self-collected in the vast majority of cases , then stored at -80□ until analysis. Of the swabs used in the final analysis, one was collected by a clinician and the collection method was missing for five samples.

Women who completed a baseline survey (including Sociodemographic data) and had a vaginal swab available were eligible for inclusion. Samples from all participants underwent Nugent scoring to determine BV status.[3,31,32] Unfortunately, vaginal pH and data regarding menstrual cycle and recent sexual activity was only available for a subset of women and were inadequate for analysis. Overall, the distribution of self-reported race/ethnicity of women in the CHOICE study were representative of the St. Louis region; few women reported a race other than “black or African-American” (hereafter referred to as “black”) or “white.” Due to small numbers of

Word Count
Abstract: 471
Main Text: 3000

other groups, only women who reported “black” or “white” race were eligible for inclusion in this sub-study.

Composition of the vaginal microbiota has been previously associated with race.[1] To test whether *Candida* was associated with vaginal niches occupied by particular bacterial communities, we sought a strategy to avoid inadequate representation of less common community types in the different demographic groups so that we would be powered to ask whether *Candida* is associated with particular microbial patterns. We used frequency matching to similarly represent black and white women in each of the three Nugent categories. We used a normal:intermediate:BV ratio of 2:2:1 to ensure that we had samples represented across the Nugent spectrum, while balancing the practical reality that relatively few BV specimens were available from white women. Of the 299 subjects selected, 35 were pregnant at the time of swab collection and excluded from final analysis. Additionally, 9 specimens were excluded due to low bacterial biomass. See Supplemental Methods.

Microbiome analysis and *Candida* colonization status:

DNA was extracted from eluted vaginal swabs and 16S ribosomal profiling of the V4 hypervariable region was performed as described in the Supplemental Methods. The microbiome was classified based on the dominant *Lactobacillus* species present, defined as 50% relative abundance or greater and referred to as, “*L. crispatus*-, *L. iners*-, *L. gasseri*-, or *L. jensenii*-dominant” microbiomes. Communities without a single *Lactobacillus* species reaching 50% were referred to as Diverse communities. A pan-*Candida* qRT-PCR[33] that amplifies the internally transcribed spacer 1 (ITS1) was used to determine *Candida* colonization status using

Word Count
Abstract: 471
Main Text: 3000

isolated DNA as template. Prior to analysis we validated this assay among vaginal specimens collected from a second cohort of women enrolled at a different site. See Supplemental Methods for details.

Candida growth inhibition:

Candida strains were grown in yeast extract-peptone-dextrose (YPD) media. *C. albicans* strain SC5314 was obtained from the American Type Culture Collection. Vaginal strains of *Candida* (*C. albicans*: BAT8133, BAT8135, BAT8143, BAT8152, BAT8154, BAT3353A; *C. glabrata*: BAT8139, BAT3353B) were isolated from women as described in the Supplemental Methods. *L. crispatus* (MV-1A-US, JV-V01, MV-3A-US, 125-2-CHN) and *L. iners* (UP II 143-D, Lactin V09V1-C, LEAF 2032-Ad, LEAF 3008-A) strains were obtained from BEI resources and cultured in De Man, Rogosa and Sharpe (MRS) media for 48 hours to make cell free supernatants (CFS). All *Candida* growth inhibition experiments were conducted in 96-well plates. Each well contained a 1:1 ratio of CFS and YPD inoculated with ~10^6 *C. albicans* colony-forming units (CFU)/mL. YPD was buffered with 300 mM sodium bicarbonate and 300 mM HEPES sodium salt for neutralization assays. For lactic acid growth inhibition assays, fresh MRS was supplemented with racemic lactic acid. A micro pH electrode was used to measure pH of each mixture and lactate was measured with a colorimetric assay. Protonated lactic acid concentrations were calculated using lactate molarity and pH using the Henderson-Hasselbalch equation (pKa = 3.9). See Supplemental Methods for more details about *Candida* growth inhibition experiments.

Statistical analysis:

Word Count
Abstract: 471
Main Text: 3000

Statistical analyses and data representation were completed in R (v3.5.1) and Prism (v7). Fisher's Exact Tests (Fisher) were used to assess for associations between cohort characteristics and race, with odds ratios (OR) determined by a conditional maximum likelihood estimate. Unless otherwise noted, we used an extension of the generalized linear model (GLM) method that included race as a potentially confounding covariate to test for associations between cohort characteristics and *Candida* colonization status, using the exponent of the coefficient from the logistic regression to calculate ORs. Note that because *Candida* colonization incidence is >10% the odds ratios may not be an accurate approximation of the relative risk; see [34] for conversion between the two.

We used type-II analysis of variance (ANOVA-II) with Wald test and Tukey's Honestly Significant Different Test (Tukey) to evaluate significance in these models. In instances where multiple statistical tests were performed, we relied on GLM accounting for race. Mann-Whitney tests were used to test for associations with *Candida* abundance and effect size (r) was calculated from the Z value. Statistical tests for *in vitro* experiments included one-way ANOVA with Tukey's correction for multiple comparisons and Mann-Whitney tests as appropriate. Regardless of the statistical method used, P-values < 0.05 were considered significant.

Results

Description of the clinical cohort:

Two-hundred fifty-five non-pregnant women of reproductive age were included in our analysis. In this cohort, 53% of women identified as "white" and 47% identified as "black". Forty-four (17%) women had BV, while 109 (43%) and 102 (40%) had intermediate and normal vaginal flora respectively. About half of the women (54%) reported using public assistance or having

Word Count
Abstract: 471
Main Text: 3000

trouble meeting daily needs and were classified as having low socioeconomic status. Body mass index (BMI) was calculated and categorized using standard methods and definitions. Most women (64.3%) reported at least one prior pregnancy. Seventy-two women (28.2%) reported vaginal douching in the last 180 days. Race was found to be associated with socioeconomic status ($p < 0.0001$), BMI ($p = 0.003$), gravidity ($p < 0.0001$) and vaginal douching ($p < 0.0001$). A summary of demographic data and cohort characteristics by race is presented in Supplemental Table 1.

Forty-two (16%) women were vaginally colonized with *Candida*. Of these, most (90%) were colonized by *C. albicans*. *C. glabrata* was less common (~10%). Sequencing of the vaginal microbiome revealed that fifty-two women (20%) had *L. crispatus*-dominant microbiomes, 99 (39%) had *L. iners*-dominant microbiomes and 98 (38%) had microbiomes that were not dominated by a single *Lactobacillus species* (Diverse). We were not powered to test associations between *Candida* and microbiomes dominated by *Lactobacillus jensenii* or *gasseri since* few women (n=6) exhibited these microbiomes. Black women were more likely than white women to have *L. iners*-dominant communities (46.7% vs 31.9% Fisher's Exact; OR = 1.87, 95% CI: 1.10 to 3.14, $p = 0.020$) and less likely to have *L. crispatus*-dominant communities (11.9% vs. 22.1% Fisher's Exact; OR = 0.380, 95% CI: 0.185 to 0.747, $p = 0.003$).

Associations between *Candida* and cohort characteristics:

Forty-two (16%) women were vaginally colonized with *Candida*. Of these, most (90%) were colonized by *C. albicans*. *C. glabrata* colonization was less common (~10%). Table 1 contains a

Word Count
Abstract: 471
Main Text: 3000

summary of *Candida* status by sociodemographic and other cohort characteristics. Only race was significantly correlated with vaginal *Candida*; black women were more likely to be colonized compared to white women (OR =2.05, 95% CI: 1.03 to 4.25, Fisher's Exact, p = 0.042). Based on these findings, race was considered to be a potential confounder and incorporated into subsequent analyses using generalized linear models (GLM) to evaluate factors associated with *Candida* colonization.

Associations between *Candida* and cohort characteristics

Candida colonization rates did not differ based on Nugent-defined BV status (GLM; ANOVA-II, p = 0.897). We did not find any association between a woman's socioeconomic status and vaginal *Candida* colonization. *Candida* colonization did not differ significantly among underweight (20% *Candida*), normal weight (18%) and overweight (23%) women. However, obese women were less likely to be colonized compared to non-obese women (GLM; OR = 0.322, 95% CI: 0.123 to 0.744; Tukey's HSD, p = 0.013, see Supplement for comment). Women reporting current use of hormonal contraceptives containing estrogen and progestin were *Candida*-colonized at higher rates than women reporting non-hormonal methods, although this did not reach statistical significance (GLM; OR = 1.77, 95% CI: 0.858 to 3.58; Tukey's HSD, p = 0.237, see Supplement for details). Women who reported vaginal douching in the last 180 days were less likely to be *Candida* positive compared to women who reported no vaginal douching (GLM; OR = 0.364, 95% CI: 0.143 to 0.838; Tukey's HSD, p = 0.047).

Relationships between *Candida* colonization and the vaginal microbiome:

Word Count
Abstract: 471
Main Text: 3000

Next, we investigated relationships between *Candida* colonization and dominant members of the vaginal microbiome based on 16S ribosomal gene profiling. *Candida* prevalence did not differ between *Lactobacillus* dominated (50% or greater *Lactobacillus*) and non-*Lactobacillus* dominated microbiomes (GLM; ANOVA-II, p = 0.327). Although the absolute abundance of *Candida* as measured by qPCR did not differ within *L. iners*-dominant communities compared to other community types (Mann-Whitney, r = 0.046, p = 0.617), *L. iners*-dominant communities were more likely to harbor *Candida* than non-*L. iners*-dominant communities (GLM; OR = 2.00, 95% CI: 1.02 to 3.98; Tukey's HSD, p = 0.045; see supplemental Table 2). Further analysis specifically showed that *L. iners*-dominant communities were more likely to be colonized than *L. crispatus*-dominant communities (OR = 2.85, 95% CI: 1.03 to 7.21; Fisher's Exact, p = 0.048). Among *Candida* positive women, higher levels of *Candida* (by qRT-PCR) were observed among black women compared to white women, although not statistically significant (Mann-Whitney test, r = 0.173, p = 0.131).

In vitro studies: inhibition of *Candida* growth by lactobacilli:

Both *L. crispatus* and lactic acid have been shown to thwart the growth of *C. albicans*.[35-37] Next, we compared the inhibitory potential of *L. crispatus* and *L. iners* on *Candida* growth *in vitro*. *C. albicans* was cultured together with cell free supernatants (CFS) from *L. crispatus* and *L. iners* (8 strains total), followed by *Candida* CFU enumeration. Compared to *L. iners* CFS, *L. crispatus* CFS resulted in lower pH (pH = 4.0 vs. pH = 4.6, p < 0.0001) and correspondingly higher levels of protonated lactic acid in CFS-YPD (55 mM vs. 11 mM, p < 0.0001) (Figure 2). Buffering CFS-YPD to a neutral pH reduced levels of protonated lactic acid to below appreciable levels, ablated *Candida* growth inhibition, and eliminated the difference in *C. albicans* growth observed

Word Count
Abstract: 471
Main Text: 3000

between *L. crispatus* and *L. iners* (Figure 2). Further, lactic acid was sufficient to inhibit *Candida* growth. In particular, significantly more growth inhibition was observed at 49 mM protonated lactic acid compared to 11 mM, levels comparable to the *L. crispatus* and *L. iners* CFS-YPD respectively. Similar findings were seen using *vaginal* isolates of *C. albicans*. In contrast, *C. glabrata* exhibited only modest growth inhibition (Figure 2). Together, these data suggest that lactic acid is both necessary and sufficient for growth inhibition of *C. albicans in vitro*.

Comment

Principal Findings: We demonstrate that *Candida* colonization is associated with characteristics of the vaginal microbiome (dominance of *L. iners* compared to *L. crispatus*). Results in clinical specimens are consistent with *in vitro* data, which show that *L. crispatus* produces a pH-dependent factor that inhibits *C. albicans* growth more effectively compared to secreted factors of *L. iners* grown under the same conditions.

Results: As a relatively common vaginal microbial community member, *Candida* may influence reproductive health. Previous studies suggested vaginal *Lactobacillus* colonization as a risk factor for *Candida* colonization or VVC,[5,6,17] but seem inconsistent with other reports of *Candida*-bacteria associations.[18-21] Here we provide more taxonomic resolution, showing that that not all *Lactobacillus*-dominant communities are equally associated with *Candida* colonization.

Clinical Implications: Clinicians often group all lactobacilli together. This study adds to the growing body of evidence suggesting that *L. iners*-dominant communities are more permissive to vaginal colonization with potential pathogens, including *Candida*.

Word Count
Abstract: 471
Main Text: 3000

Research Implications: Of interest, black race was associated with obesity and vaginal douching as in prior studies. But surprisingly, the correlation between *Candida* and black race cannot be accounted for by obesity or douching because obese women and those who douche were actually ***less*** likely to be colonized with *Candida* (OR = 0.322 and 0.364 respectively). The literature contains inconsistent reports regarding the role of *Lactobacillus* colonization as a risk factor for *Candida* colonization or VVC.[5,6,17,18-21] We show that that not all *Lactobacillus*-dominant communities are equally associated with *Candida*. *In vitro* data provide one possible explanation, showing that *L. iners* strains do not produce the same magnitude of lactic acid compared to *L. crispatus* strains. An alternative, albeit not mutually exclusive explanation, is that vaginal *Candida* colonization may shift the microbiome to favor *L. iners*.

Interestingly, we observed similar rates of *Candida* colonization in *L. crispatus*-dominant and Diverse communities. With fewer lactic acid producing bacteria present, the vaginal pH of women with Diverse microbiome is less acidic.[1] These findings indicate that Diverse communities resist *Candida* by lactic acid-independent mechanisms.

Additional studies are needed to evaluate potential mechanisms governing these relationships and apply these findings in clinical settings.

Strengths and Limitations: Key strengths of our study design were the validation of a *Candida*-specific qPCR assay[33] for laboratory testing for *Candida* colonization, offering flexibility in settings where archived frozen vaginal swabs are more practical. We acknowledge that the specimens selected for this study are not a naturalistic representation of vaginal microbiomes. Rather, the frequency matching of black and white women across the Nugent spectrum is a strength that enabled power to test associations between yeast and bacteria in different racial groups. Limitations include: 1) the sample size and number of *Candida*-positive women were

Word Count
Abstract: 471
Main Text: 3000

relatively small, limiting power to model multiple potential confounders, 2) this cohort may not be representative of the U.S. population, 3) clinical data were not available to examine the relationship between *Candida* colonization and VVC, and 4) our *in vitro* findings may not be representative of *in vivo* relationships.

Conclusion: These data suggest that *L. iners*-dominant vaginal communities may support the co-occurrence of *Candida.*

.

Acknowledgements: We thank the Contraceptive CHOICE Project support staff and Jennifer Reed (née Bick, Washington University) for Nugent scoring. We appreciate Dr. Sharon Hillier for verifying Nugent scoring. We also thank Denise Spear WHNP-BC, Valerie Higginbotham WHNP-BC (St. Louis County Department of Public Health) and Courtney Amegashie (Washington University, NIH funding) for collection and swab processing and the St. Louis County Department of Public Health for facilitating sample collection used to validate the *Candida* qPCR assay. We thank Andrew Kau for advice (Washington University, in part NIH funding) on microbiome profiling and Deborah Frank (Washington University) for editorial input. Finally, we appreciate those who provided vaginal samples.

Word Count
Abstract: 471
Main Text: 3000

References:

1. Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A.* 2011;108 Suppl 1:4680-4687.
2. Fettweis JM, Brooks JP, Serrano MG, et al. Differences in vaginal microbiome in African American women versus women of European ancestry. *Microbiology.* 2014;160(Pt 10):2272-2282.
3. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. *J Clin Microbiol.* 1991;29(2):297-301.
4. Anahtar MN, Gootenberg DB, Mitchell CM, Kwon DS. Cervicovaginal Microbiota and Reproductive Health: The Virtue of Simplicity. *Cell Host Microbe.* 2018;23(2):159-168.
5. Beigi RH, Meyn LA, Moore DM, Krohn MA, Hillier SL. Vaginal yeast colonization in nonpregnant women: a longitudinal study. *Obstet Gynecol.* 2004;104(5 Pt 1):926-930.
6. Cotch MF, Hillier SL, Gibbs RS, Eschenbach DA. Epidemiology and outcomes associated with moderate to heavy *Candida* colonization during pregnancy. Vaginal Infections and Prematurity Study Group. *Am J Obstet Gynecol.* 1998;178(2):374-380.
7. Brandolt TM, Klafke GB, Goncalves CV, et al. Prevalence of *Candida* spp. in cervical-vaginal samples and the *in vitro* susceptibility of isolates. *Braz J Microbiol.* 2017;48(1):145-150.
8. Payne MS, Ireland DJ, Watts R, et al. *Ureaplasma parvum* genotype, combined vaginal colonisation with *Candida albicans*, and spontaneous preterm birth in an Australian cohort of pregnant women. *BMC Pregnancy Childbirth.* 2016;16(1):312.

Word Count
Abstract: 471
Main Text: 3000

9. Payne MS, Cullinane M, Garland SM, et al. Detection of *Candida* spp. in the vagina of a cohort of nulliparous pregnant women by culture and molecular methods: Is there an association between maternal vaginal and infant oral colonisation? *Aust N Z J Obstet Gynaecol.* 2016;56(2):179-184.

10. Fidel PL, Jr., Barousse M, Espinosa T, et al. An intravaginal live *Candida* challenge in humans leads to new hypotheses for the immunopathogenesis of vulvovaginal candidiasis. *Infect Immun.* 2004;72(5):2939-2946.

11. Maki Y, Fujisaki M, Sato Y, Sameshima H. *Candida* Chorioamnionitis Leads to Preterm Birth and Adverse Fetal-Neonatal Outcome. *Infect Dis Obstet Gynecol.* 2017;2017:9060138.

12. To V, Gurberg J, Krishnamurthy S. Tubo-Ovarian Abscess Caused by *Candida albicans* in an Obese Patient. *J Obstet Gynaecol Can.* 2015;37(5):426-429.

13. Menon S, Broeck DV, Rossi R, Ogbe E, Harmon S, Mabeya H. Associations Between Vaginal Infections and Potential High-risk and High-risk Human Papillomavirus Genotypes in Female Sex Workers in Western Kenya. *Clin Ther.* 2016;38(12):2567-2577.

14. Holzer I, Farr A, Kiss H, Hagmann M, Petricevic L. The colonization with *Candida* species is more harmful in the second trimester of pregnancy. *Arch Gynecol Obstet.* 2017;295(4):891-895.

15. Farr A, Kiss H, Holzer I, Husslein P, Hagmann M, Petricevic L. Effect of asymptomatic vaginal colonization with *Candida albicans* on pregnancy outcome. *Acta Obstet Gynecol Scand.* 2015;94(9):989-996.

Word Count
Abstract: 471
Main Text: 3000

16. Babu G, Singaravelu BG, Srikumar R, Reddy SV, Kokan A. Comparative Study on the Vaginal Flora and Incidence of Asymptomatic Vaginosis among Healthy Women and in Women with Infertility Problems of Reproductive Age. *J Clin Diagn Res.* 2017;11(8):DC18-DC22.

17. McClelland RS, Richardson BA, Hassan WM, et al. Prospective study of vaginal bacterial flora and other risk factors for vulvovaginal candidiasis. *J Infect Dis.* 2009;199(12):1883-1890.

18. Gonzalez-Fernandez D, Koski KG, Sinisterra OT, Del Carmen Pons E, Murillo E, Scott ME. Interactions among urogenital, intestinal, skin, and oral infections in pregnant and lactating Panamanian Ngabe women: a neglected public health challenge. *Am J Trop Med Hyg.* 2015;92(6):1100-1110.

19. Liu MB, Xu SR, He Y, et al. Diverse vaginal microbiomes in reproductive-age women with vulvovaginal candidiasis. *PLoS One.* 2013;8(11):e79812.

20. Vahidnia A, Tuin H, Bliekendaal H, Spaargaren J. Association of sexually transmitted infections, *Candida* species, Gram-positive flora and perianal flora with bacterial vaginosis. *New Microbiol.* 2015;38(4):559-563.

21. Rathod SD, Klausner JD, Krupp K, Reingold AL, Madhivanan P. Epidemiologic features of Vulvovaginal Candidiasis among reproductive-age women in India. *Infect Dis Obstet Gynecol.* 2012;2012:859071.

22. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. *N Engl J Med.* 2005;353(18):1899-1911.

23. Fredricks DN. Molecular methods to describe the spectrum and dynamics of the vaginal microbiota. *Anaerobe.* 2011;17(4):191-195.

Word Count
Abstract: 471
Main Text: 3000

24. Rampersaud R, Lewis EL, LaRocca TJ, Ratner AJ. Environmental pH modulates inerolysin activity via post-binding blockade. *Sci Rep.* 2018;8(1):1542.

25. Rampersaud R, Planet PJ, Randis TM, et al. Inerolysin, a cholesterol-dependent cytolysin produced by *Lactobacillus iners*. *J Bacteriol.* 2011;193(5):1034-1041.

26. van Houdt R, Ma B, Bruisten SM, Speksnijder A, Ravel J, de Vries HJC. *Lactobacillus iners*-dominated vaginal microbiota is associated with increased susceptibility to *Chlamydia trachomatis* infection in Dutch women: a case-control study. *Sex Transm Infect.* 2018;94(2):117-123.

27. Muzny CA, Blanchard E, Taylor CM, et al. Identification of Key Bacteria Involved in the Induction of Incident Bacterial Vaginosis: A Prospective Study. *J Infect Dis.* 2018;218(6):966-978.

28. Nunn KL, Wang YY, Harit D, et al. Enhanced Trapping of HIV-1 by Human Cervicovaginal Mucus Is Associated with *Lactobacillus crispatus*-Dominant Microbiota. *MBio.* 2015;6(5):e01084-01015.

29. Joag V, Obila O, Gajer P, et al. Impact of Standard Bacterial Vaginosis Treatment on the Genital Microbiota, Immune Milieu, and Ex Vivo Human Immunodeficiency Virus Susceptibility. *Clin Infect Dis.* 2018.

30. Secura GM, Allsworth JE, Madden T, Mullersman JL, Peipert JF. The Contraceptive CHOICE Project: reducing barriers to long-acting reversible contraception. *Am J Obstet Gynecol.* 2010;203(2):115 e111-117.

31. Lewis WG, Robinson LS, Perry J, et al. Hydrolysis of secreted sialoglycoprotein immunoglobulin A (IgA) in ex vivo and biochemical models of bacterial vaginosis. *J Biol Chem.* 2012;287(3):2079-2089.

Word Count
Abstract: 471
Main Text: 3000

32. Amegashie CP, Gilbert NM, Peipert JF, Allsworth JE, Lewis WG, Lewis AL. Relationship between nugent score and vaginal epithelial exfoliation. *PLoS One.* 2017;12(5):e0177797.

33. Zhang J, Hung GC, Nagamine K, Li B, Tsai S, Lo SC. Development of *Candida*-Specific Real-Time PCR Assays for the Detection and Identification of Eight Medically Important *Candida* Species. *Microbiol Insights.* 2016;9:21-28.

34. Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. *JAMA.* 1998;280(19):1690-1691.

35. Krasner RI, Young G, Yudkofsky PL. Interactions of oral strains of *Candida albicans* and lactobacilli. *J Bacteriol.* 1956;72(4):525-529.

36. Wang S, Wang Q, Yang E, Yan L, Li T, Zhuang H. Antimicrobial Compounds Produced by Vaginal *Lactobacillus crispatus* are able to Strongly Inhibit *Candida albicans* Growth, Hyphal Formation and Regulate Virulence-related Gene Expressions. *Front Microbiol.* 2017;8:564.

37. Parolin C, Marangoni A, Laghi L, et al. Isolation of Vaginal lactobacilli and Characterization of Anti-*Candida* Activity. *PLoS One.* 2015;10(6):e0131220.

38. Kau AL, Planer JD, Liu J, et al. Functional characterization of IgA-targeted bacterial taxa from undernourished Malawian children that produce diet-dependent enteropathy. *Sci Transl Med.* 2015;7(276):276ra224.

39. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science.* 2013;341(6150):1241214.

Word Count
Abstract: 471
Main Text: 3000

40. Koumans EH, Sternberg M, Bruce C, et al. The prevalence of bacterial vaginosis in the United States, 2001-2004; associations with symptoms, sexual behaviors, and reproductive health. *Sex Transm Dis.* 2007;34(11):864-869.

41. Brookheart RT, Lewis WG, Peipert JF, Lewis AL, Allsworth JE. Association between obesity and bacterial vaginosis as assessed by Nugent score. *Am J Obstet Gynecol.* 2019.

42. Si J, You HJ, Yu J, Sung J, Ko G. *Prevotella* as a Hub for Vaginal Microbiota under the Influence of Host Genetics and Their Association with Obesity. *Cell Host Microbe.* 2017;21(1):97-105.

43. Marrazzo JM, Fiedler TL, Srinivasan S, et al. Extravaginal reservoirs of vaginal bacteria as risk factors for incident bacterial vaginosis. *J Infect Dis.* 2012;205(10):1580-1588.

Word Count
Abstract: 471
Main Text: 3000

Table 1: Characteristics of subjects with vaginal *Candida* compared with those without vaginal *Candida.*

Characteristics	Total Cohort	*Candida* Positive	*Candida* Negative	P-value
Total Number of Subjects	255	42 (16.5)	213 (83.5)	
Age				0.811
< 20	28 (11.0)	6 (14.3)	22 (10.3)	
20 to 29	178 (69.8)	29 (69.0)	149 (70.0)	
30 to 39	44 (17.3)	7 (16.7)	37 (17.4)	
40 +	5 (2.0)	0 (0.0)	5 (2.3)	
Race				0.042
Black	120 (47.1)	26 (61.9)	94 (44.1)	
White	135 (52.9)	16 (38.1)	119 (55.9)	
Nugent-defined Vaginal Flora				0.833
Normal	102 (40.0)	15 (35.7)	87 (40.8)	
Intermediate	109 (42.7)	19 (45.2)	90 (42.3)	
BV	44 (17.3)	8 (19.0)	36 (16.9)	
Socioeconomic Status (SES)				1

Word Count
Abstract: 471
Main Text: 3000

Low SES	138 (54.1)	23 (54.8)	115 (54.0)	
Not Low SES	117 (45.9)	19 (45.2)	98 (46.0)	
Body Mass Index (kg/m2)				0.127
Underweight (< 18.5)	15 (5.9)	3 (7.1)	12 (5.6)	
Normal Weight (18.5 - 24.9)	103 (40.4)	19 (45.2)	84 (39.4)	
Overweight (25 - 30)	48 (18.8)	11 (26.2)	37 (17.4)	
Obese (> 30)	78 (30.6)	7 (16.7)	71 (33.3)	
Not Documented	11 (4.3)	2 (4.8)	9 (4.2)	
Current Birth Control Method				0.320
Estrogen + Progestin[a]	72 (28.2)	16 (38.1)	56 (26.3)	
Progestin[b]	12 (4.7)	1(2.4)	11 (5.2)	
Non-Hormonal[c]	171 (67.1)	25 (59.5)	146 (68.5)	
Vaginal Douching in Last 180 Days				0.323
Yes	72 (28.2)	8 (19.0)	64 (30.0)	
No	182 (71.4)	34 (81.0)	148 (69.5)	
Don't Know	1 (0.4)	0 (0.0)	1 (0.5)	
Gravidity				0.160
None	91 (35.7)	15 (35.7)	76 (35.7)	

Word Count
Abstract: 471
Main Text: 3000

1	58 (22.7)	6 (14.3)	52 (24.4)	
2	47 (18.4)	6 (14.3)	41 (19.2)	
3+	59 (23.1)	15 (35.7)	44 (20.7)	
Community Type				0.113
L. crispatus-dominant	52 (20.4)	5 (11.9)	47 (22.1)	
L. iners-dominant	99 (38.8)	23 (54.8)	76 (35.7)	
L. jensenii-dominant	3 (1.2)	1 (2.4)	2 (0.9)	
L. gasseri-dominant	3 (1.2)	0 (0.0)	3 (1.4)	
Diverse	98 (38.4)	13 (31.0)	85 (39.9)	

Values are n (%). Fisher's Exact Tests were used to determine p-values for each set of variables without adjusting for race. Note that p-values given in the text use GLM (accounting for race as a potential confounder).

[a]Women who reported the oral contraceptive pill or the birth control ring;

[b]Women who reported the levonorgestrel-containing intrauterine device or depot medroxyprogesterone acetate;

[c]Women who reported condoms, rhythm/natural family planning, abstinence, withdrawal or nothing.

Word Count
Abstract: 471
Main Text: 3000

Figure 1: Heatmap of all samples in the cohort clustered by community type.

Heat map of samples clustered by community type showing the top 25 taxa observed across the cohort. The bars above the heatmap indicate community type, BV status by Nugent score, race and *Candida* status. In the heat map, *light blue* indicates the highest abundance, *darker blues* indicate lower abundance and *black* indicates very low abundance or not present. Black race ($p = 0.037$) and *L. iners*-dominant communities ($p = 0.045$) were associated with *Candida* colonization.

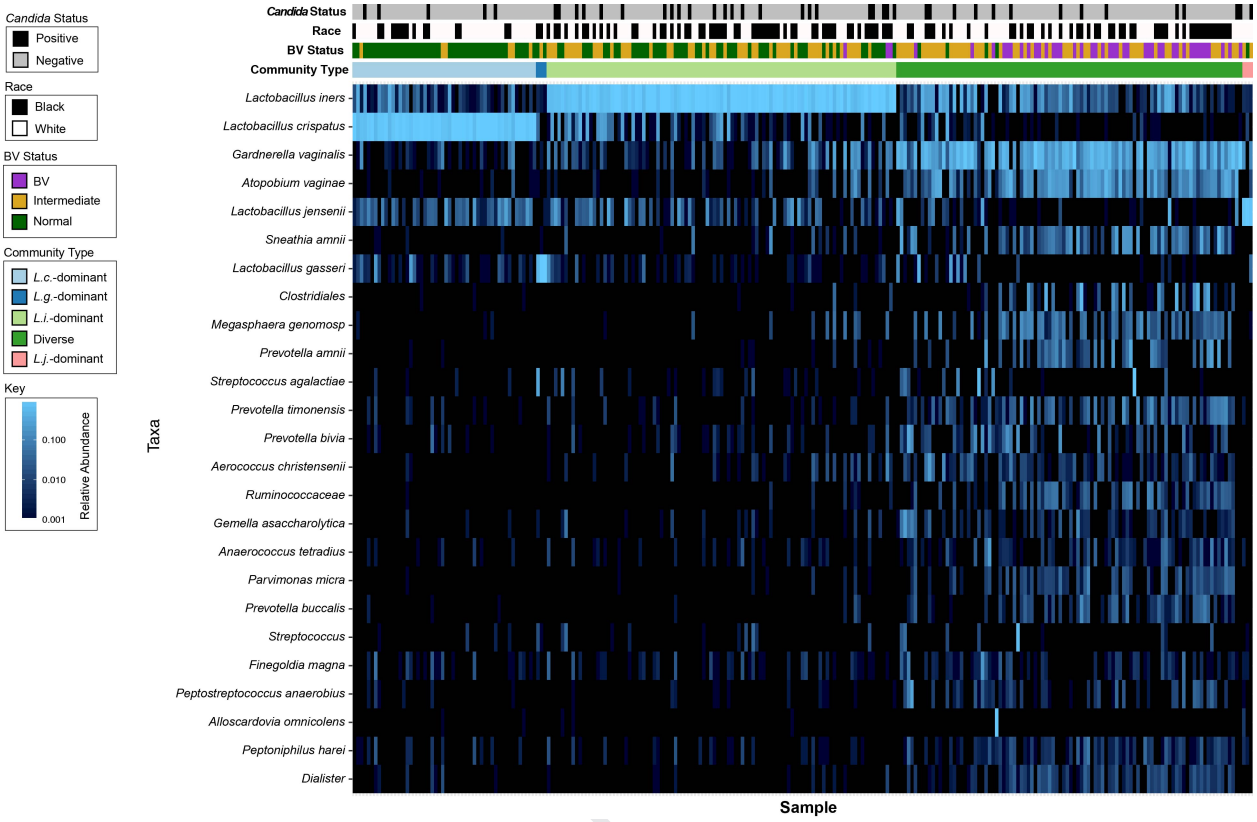
Figure 2: *In vitro* inhibition of *Candida* by *Lactobacillus* CFS and lactic acid.

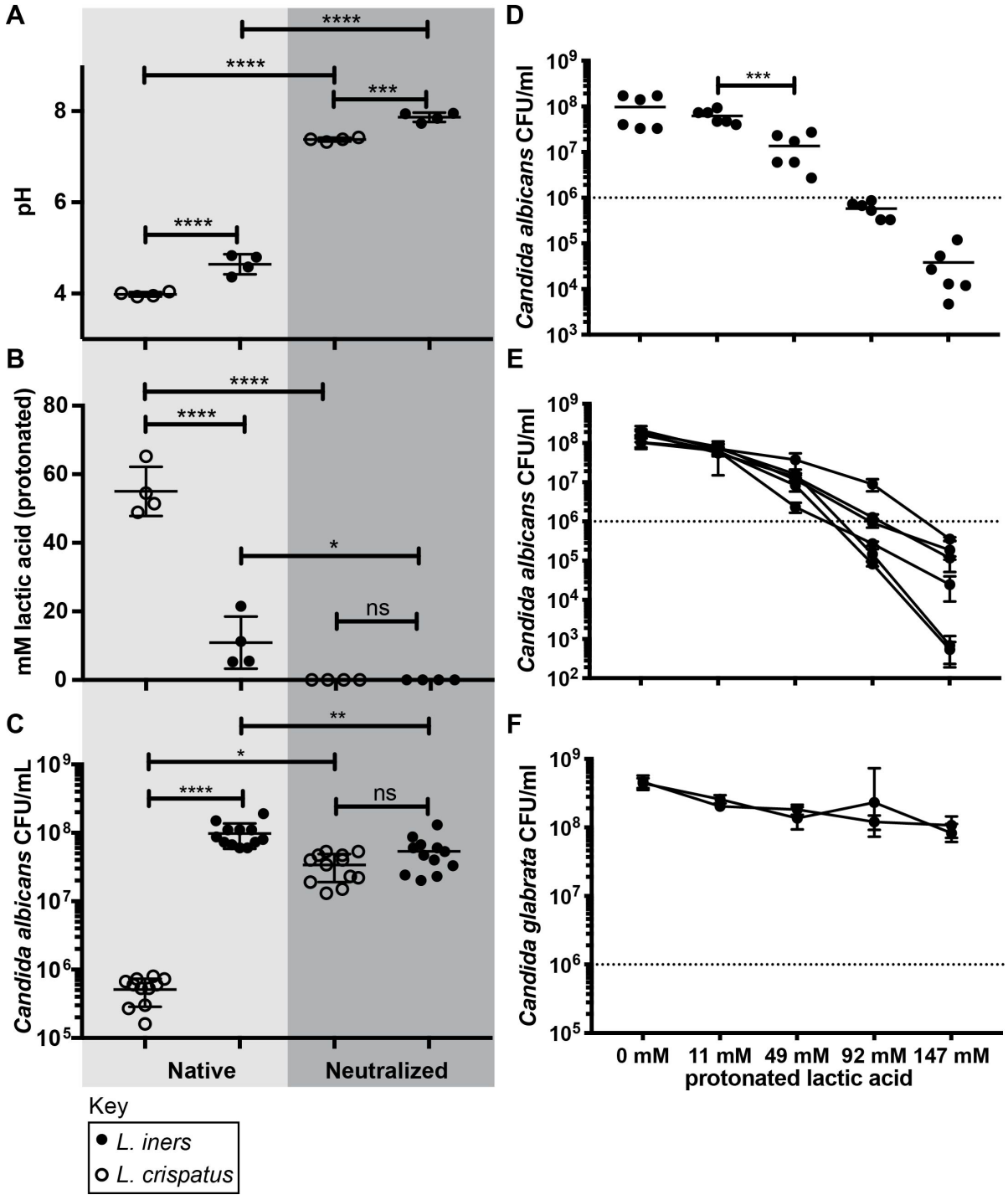
A-B, Characterization of *Candida* growth medium supplemented with *Lactobacillus* CFS (YPD-CFS) in native and buffered states from four *L. crispatus* and four *L. iners* strains, prior to *Candida* inoculation. **A**, pH of YPD-CFS; **B**, Concentration of protonated lactic acid in YPD-CFS; **C,** Growth inhibition of *Candida* laboratory strain SC5314, showing three technical replicates for each *Lactobacillus* YPD-CFS. Analysis by one-way ANOVA with Tukey's correction for multiple comparisons. **D-F**, Characterization of the inhibitory effect of lactic acid supplemented medium on *Candida* growth. Three technical replicates from two biological experiments are shown. **D**, Growth inhibition of SC5314 by lactic acid showing Mann-Whitney test comparison of 11 mM to 49 mM protonated lactic acid; **E**, Lactic acid growth inhibition of 6 vaginal *C. albicans* isolates; **F**, Lactic acid growth inhibition of 2 vaginal *C. glabrata* isolates. Data points in panel D reflect 6 replicates from two experiments for each condition. Error bars in E-F show the standard deviation from the mean of three replicates for each isolate. Approximate

Word Count
Abstract: 471
Main Text: 3000

starting inoculum for growth assays is indicated by a dashed line. Statistical significance: ns (not significant), *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.0001.

Word Count
Abstract: 471
Main Text: 3000




A
pH
B
mM lactic acid (protonated)
C
Candida albicans CFU/mL
Native
Neutralized
D
Candida albicans CFU/ml
E
Candida albicans CFU/ml
F
Candida glabrata CFU/ml
0 mM
11 mM
49 mM
92 mM
147 mM
protonated lactic acid
Key
L. iners
L. crispatus

Supplemental Information for:

Associations between the vaginal microbiome and *Candida* colonization in women of reproductive age

Mr. Brett A. TORTELLI B.A.[1,2], Warren G. LEWIS Ph.D.[2], Jenifer E. ALLSWORTH Ph.D.[6], Mr. Nadum MEMBER-MENEH B.S.[2,3], Ms. Lynne R. FOSTER B.S.[2,3], Hilary E. RENO M.D. Ph.D.[4], Jeffrey F. PEIPERT M.D., Ph.D. [7], Justin C. FAY* Ph.D.[1,8], Amanda L. LEWIS* Ph.D.[2,3,5]

Author affiliations:
[1]Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA.
[2]Center for Women's Infectious Disease Research, Washington University School of Medicine, St. Louis, MO, USA.
[3]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA.
[4]Division of Infectious Diseases, Washington University School of Medicine, St. Louis, MO 63110, USA.
[5]Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO 63110, USA.
[6]Department of Biomedical and Health Informatics, University of Missouri-Kansas City School of Medicine, Kansas City, MO, USA.
[7]Department of Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN, USA.
[8]Department of Biology, University of Rochester, Rochester, NY, USA.

Supplemental Methods

Coding Survey Data: Data pertaining to age, socioeconomic status (SES), body mass index (BMI), current birth control method, vaginal douching and gravidity were extracted from survey response data and categorized. Age in years was converted to the following categorical variable: less than 20, 20 to 29, 30 to 39, and 40 or more. Low socioeconomic status (Low SES) was defined as reporting any current receipt of public assistance (food stamps; Special Supplemental Nutrition Program for Women, Infants and Children; welfare; or unemployment) or trouble paying for necessities (transportation, housing, health or medical care, or food). BMI (kg/m^2)

was converted to categorical variables as follows: underweight (< 18.5), normal weight (18.5 to 24.9), overweight (25-30) and obese (>30). Current birth control method was categorized into one of three categories: hormonal contraceptives containing a combination of estrogen and progestin (Estrogen + Progestin), hormonal contraceptives containing progestin alone (Progestin), or non-hormonal contraceptive methods (Non-hormonal).

Responses indicating the use of oral contraceptive pills or birth control ring were grouped as "Estrogen + Progestin". Responses indicating the use of a levonorgestrel containing intrauterine device or depot medroxyprogesterone acetate were grouped as "Progestin". Responses indicating the use of condoms, rhythm/natural family planning method, abstinence, withdrawal or nothing were classified as "Non-hormonal". The number of times a patient reported vaginal douching in the past 180 days was converted to a categorical variable: "yes" if the number was 1 or more, "no" if it was 0 and "don't know" if the patient reported not knowing. Gravidity was converted to a categorical variable with "3+" designating a response of 3 or more.

Vaginal Swab Processing and Controls:

Frozen vaginal swabs (CHOICE study) were arrayed in deep well 96 well plates (Eppendorf, Hauppauge, NY) in 1 mL of 0.1 M sodium acetate buffer (pH 5.5). To minimize cross-contamination during swab elution, each 96 well plate of swabs was arrayed on two plates in a checkerboard fashion, such that empty wells were present between samples. Swabs were incubated for one hour on ice and agitated every 20 minutes manually. Swabs were removed, and the two plates merged by transferring suspensions into a single deep well 96 well plate. The plate was then centrifuged at 32,000 x g at 4℃ for 20 minutes and the supernatants removed from the samples. The pelleted material was resuspended in 250 microliters of a buffer containing 200

mM Tris-HCl (pH 8.0), 200 mM NaCl and 20 mM EDTA and then transferred to a 2 mL screwcap tube (Axygen, Oneonta, NY) containing 250 µL of (0.1 mm) zirconia/silica beads and 105 µL of 20% SDS. 250 µL of a solution of phenol:chloroform:isoamyl alcohol (25:24:1) saturated with 10 mM Tris (pH 8.0) and 1mM EDTA (Sigma-Aldrich, St. Louis, MO) was added. Samples were lysed by mechanical disruption with a bead beater (Biospec Products, Bartelsville, OK) for 3 minutes at room temperature before being centrifuged at 32,000 x g at 4℃ for 5 minutes. DNA was cleaned and concentrated from the aqueous layer using a QIAquick 96-well PCR Purification Kit (Qiagen, Germantown, MD) with some modifications to the manufacturer instructions. The extraction process was automated with an EPMotion that performed all pipetting steps. The binding buffer was modified by supplementing 500 mL of Buffer PM with 33.3 mL of 3M sodium acetate (pH 5.5). DNA was eluted from the columns with 50 µL of water into 96-well PCR plates (Phenix Research Products, Candler, NC). Each 96-well plate of samples contained the following reagent controls: eight wells of sodium acetate-eluted sterile swabs and eight wells of sodium acetate buffer used for swab elution. DNA was normalized to 5 ng/µL and all samples diluted 1:5 after normalization to dilute PCR inhibitors. All PCR plates were sealed with Biomek aluminum foil seals (Becker Coulter, Brea, CA). To avoid cross-contamination, plates were centrifuged at 32,000 x g prior to removal of the seal and resealed after each use. Also, caution was exercised when using a multichannel pipettor to mix samples, microscale splashes and aerosol that could cause cross-contamination were avoided by gentle pipetting and expelling material only to the soft stop.

16S Sequencing:

The V4 hypervariable region was PCR amplified by adding 6.4 μL normalized genomic DNA (dilution process described above) to a PCR master mix containing primers with integrated barcodes for multiplexing as previously described.[38] PCR product was then quantified with a Quant-iT dsDNA Assay (Invitrogen, Carlsbad, CA) and pooled into quartiles based on abundance prior to size selection by AMPure XP magnetic beads (Beckman Coulter, Pasadena, CA). Each purified quartile was then quantified and pooled into a library for 2 x 250 paired-end sequencing on an Illumina MiSeq platform through the Center for Genome Science at Washington University in St. Louis.

Microbiome Analysis:

Reads were trimmed to a length of 200 base pairs and mate-pairs merged with a minimum overlap of 18 bases. All analysis with Qiime software was completed with version 1.9.0. Reads were demultiplexed and OTUs clustered as previously described.[38] Taxonomy was assigned to OTUs using RDP 2.4 trained on a custom database as previously described.[39] Taxa were assigned with a confidence of 0.7 or greater. Because the V4 region among some common vaginal *Lactobacillus species* (i.e. *Lactobacillus crispatus*) share high sequence similarity with other *Lactobacillus species* that rarely colonize the vagina, a modified approach to classifying *Lactobacillus* OTUs to the species level was completed. OTUs assigned to the genus *Lactobacillus* were aligned to the NCBI 16S database using BLASTn. The top ranked species returned with a sequence homology of 97% or greater was identified as the OTU species. If the top BLASTn hit was less than 97% identical, the OTU was not assigned to the species. Read data was then rarefied so that each sample contained 1000 reads.

Inclusion Criteria for Analysis:

Low bacterial biomass samples are at increased risk of having endogenous signal masked by contamination. To avoid the inclusion of low bacterial biomass samples, we used the abundance of the V4 amplicon after 16s PCR as a proxy of bacterial biomass. V4 amplicon abundance was quantified after 16s PCR and reagent control samples were used to determine the threshold for inclusion. The maximum V4 amplicon concentration from all 64 reagent controls quantified was chosen as the cutoff for inclusion in analysis (6.1 ng/μL). We removed 9 samples from analysis due to low V4 amplicon abundance.

***Candida* qPCR Validation:**

A separate cohort was needed to validate the qPCR assay (see below) we later used for determining *Candida* colonization status. Women were recruited from the North Central Community Health Center according to Washington University IRB-approved protocol number 201704121. Women underwent a speculum exam by a clinician, during which mid-vaginal swabs were collected. Two double-headed anaerobic swabs were collected and transported using the Starswab Anaerobic Transport System (Starplex Scientific Inc, Cleveland, TN). Two standard aerobic Starplex double headed rayon swabs (S09D, Starplex Scientific Inc, Cleveland, TN) were also collected. Anaerobic swabs were transported to the laboratory for same day processing and aerobic swabs frozen at -80℃. Anaerobic swabs were eluted in 2X NYCIII media, and "fresh frozen" (i.e. "0 passage," without growth or amplification of any kind) in the presence of sterile glycerol (20% final). Aliquots of fresh frozen material were then stored at -80℃. Fresh frozen aliquots were thawed on ice and centrifuged at 3,000 x g for 5 minutes. The media was removed, the pellet resuspended in 200 μL of YPD, and plated on CHROMagar *Candida* semi-selective plates (DRG, Springfield,

NJ). Plates were incubated for 48 hours aerobically at 37℃. Plates were then examined and specimens were considered to be culture positive if *Candida* colonies distinguished by a distinctive green color were observed. Specimens that were culture positive for *Candida* were considered to be true positives and this information was used to determine the sensitivity and specificity of the *Candida* (ITS1) qPCR assay described below (conducted on DNA that was isolated from the eluted aerobic swab).

Determination of *Candida* Colonization Status:

A pan-*Candida* qPCR[33] designed to detect medically relevant *Candida* species in the presence of human genomic DNA, was validated for use on DNA extracted from vaginal swabs as described above. The primers 18S-1F (GCAAGTCATCAGCTTGCGTT) and 5.8S-1R (TGCGTTCTTCATCGATGCGA) amplify the internally transcribed spacer 1 (ITS1). Power SYBR Green PCR Master Mix (Applied Biosystems, Waltham, MA) was used for the qPCR reaction and each reaction contained 2 ng of genomic DNA as template. All reactions were run in triplicate. CT values were converted to ng of *Candida* DNA based off a standard curve of genomic DNA extracted from *C. albicans* strain SC5314. A sample was denoted as "*Candida* Positive" if the mean of the replicates was one standard deviation greater than the reported detection limit of 10 fg of *Candida* DNA. Candida DNA quantities were adjusted for initial genomic DNA normalization to 5 ng/uL and used as a proxy for *Candida* abundance. *Candida species* identification was confirmed by Sanger sequencing the ITS1 amplicon and BLASTing the sequence against the NCBI database.

***Candida* Strains:**

The *C. albicans* strain SC5314 was obtained from the American Type Culture Collection in Manassas, VA. Vaginal *Candida* strains that were used in the *in vitro* assays were isolated from vaginal swab specimens originally collected from pregnant women as part of a different study, in accordance with Washington University IRB-approved protocol number 201610121. Vaginal swabs were rolled on CHROMagar *Candida* plates to isolate *Candida* colonies and species identification was confirmed by sequencing.

Preparation of *Lactobacillus* Cell Free Supernatant (CFS):

Four strains of *L. crispatus* (MV-1A-US, JV-V01, MV-3A-US, 125-2-CHN) and four strains of *L. iners* (UP II 143-D, Lactin V09V1-C, LEAF 2032-Ad, LEAF 3008-A) were cultured to make CFS. *Lactobacilli* were grown in 10 mL of De Man, Rogosa and Sharpe (MRS) media (pH 6.5) for 48 hours in 10 mL cell culture flasks (GBO, Monroe, NC) at 37℃ in an anaerobic chamber (Coy, Grass Lake, MI). Cultures were centrifuged at 3200 x g for 20 minutes at 4℃ and the supernatants filtered through a 0.22 μm filter to remove residual bacteria. CFS was aliquoted in microcentrifuge tubes and stored at -20℃. Our findings were reproduced with two different batches of CFS from *L. crispatus* and *L. iners.*

CFS Growth Inhibition Assays:

All CFS growth inhibition experiments were conducted in 96-well microplates (GBO, Monroe, NC). A mixture of 50 μL *Lactobacillus* CFS, 40 μL YPD, and 10 μL *C. albicans* suspension, ~10_6 colony-forming units (CFU)/mL, were added to each well. Unconditioned MRS media was added instead of *Lactobacillus* CFS as a control. The plates were sealed with breathable seals (Diversified Biotech, Dedham, MA) and incubated aerobically for 16 hours at 37℃ with constant

shaking at 300 rpm. Suspensions were then plated for CFU on YPD agarose plates. For CFS neutralization assay, YPD was buffered to by adding 300 mM sodium bicarbonate and 300 mM HEPES resulting in a final pH of 8.6. Lactate concentrations of CFS supplemented YPD medium were measured with a colorimetric assay adapted for microplate use (Megazyme, Chicago, IL). A micro pH meter (S220-MIC, Mettler-Toledo, Columbus, OH) was used to determine the pH of each mixture and the protonated lactic acid concentration calculated using lactate molarity and pH using the Henderson-Hasselbalch equation (pKa = 3.9). Each growth inhibition experiment was conducted in triplicate and repeated at least twice.

Lactic Acid Inhibition Assays:

MRS was supplemented with racemic lactic acid (Sigma-Aldrich, St. Louis, MO) at the following final concentrations: 100 mM, 200 mM, 300 mM and 400 mM. A mixture of 50 µL lactic acid supplemented MRS, 40 µL YPD, and 10 µL *Candida* suspension (~10_7 CFU/mL) were added to each well. Fresh non-conditioned MRS media was added instead of lactic acid supplemented MRS as a control. Suspensions were then plated for CFU on YPD agarose plates. Lactate concentrations and pH of lactic acid supplemented YPD medium were measured as described above and used to determine the protonated lactic acid concentration. Each growth experiment was conducted in triplicate and repeated at least twice.

Supplemental Comment

The effect of obesity on the composition of the vaginal microbiome is not well understood. Recent studies showed that Nugent score was positively associated with BMI[40,41] and obesity has

also been associated with greater overall diversity and colonization by particular BV-associated taxa.[42] Previously it was found that obese women were less likely to be heavily colonized with *Candida*.[6] Our data support the same conclusion, although using a much more sensitive detection method. We found that regardless of race, obese women were more likely to have *L.i.*-dominant than *L.c.*-dominant communities. Taken together with our finding that *L.i.*-dominant communities were more likely than *L.c.*-dominant communities to harbor Candida, the data suggest a more complex and multifactorial interaction that cannot be explained by the dominant species of *Lactobacillus* present in the vagina. Further study is required to understand the interplay between obesity, the microbiome and *Candida* colonization. Factors that could contribute to this interplay may include disturbances in host metabolic, hormonal, and/or immune function associated with obesity. A higher prevalence of menstrual irregularity in obese women could also contribute to changes in the microbiome. Behaviors could also play a role, for example, obese women may be more likely to engage in vaginal douching.[40,41] Previous links between the gut microbiome and obesity could also be involved, especially given findings that the gut microbiome can be a reservoir of vaginal community members,[43] including *Candida*.

Supplemental Table 1: Characteristics of subjects of black race compared with those of white race

Characteristics	Total Cohort	White Race	Black Race	P-value
Total Number of Subjects	255	135 (52.9)	120 (47.1)	
Age				0.3073
< 20	28 (11.0)	12 (8.9)	16 (13.3)	

20 to 29	178 (69.8)	101 (74.8)	77 (64.2)	
30 to 39	44 (17.3)	20 (14.8)	24 (20.0)	
40 +	5 (2.0)	2 (1.5)	3 (2.5)	
Nugent-defined Vaginal Flora				0.420
BV	44 (17.3)	21 (15.6)	23 (19.2)	
Intermediate	109 (42.7)	55 (40.7)	54 (45.0)	
Normal	102 (40.0)	59 (43.7)	43 (35.8)	
Socioeconomic Status (SES)				< 0.0001
Not Low SES	117 (45.9)	82 (60.7)	35 (29.2)	
Low SES	138 (54.1)	53 (39.3)	85 (70.8)	
Body Mass Index (kg/m2)				0.003
Underweight (< 18.5)	15 (5.9)	9 (6.7)	6 (5.0)	
Normal Weight (18.5 - 24.9)	103 (40.4)	68 (50.4)	35 (29.2)	
Overweight (25 - 30)	48 (18.8)	22 (16.3)	26 (21.7)	
Obese (> 30)	78 (30.6)	31 (23.0)	47 (39.2)	
Not Documented	11 (4.3)	5 (3.7)	6 (5.0)	
Current Birth Control Method				0.108
Estrogen + Progestin[a]	72 (28.2)	42 (31.1)	30 (25.0)	
Progestin[b]	12 (4.7)	3 (2.2)	9 (7.5)	
Non-Hormonal[c]	171 (67.1)	90 (66.7)	81 (67.5)	
Vaginal Douching in Last 180 Days				< 0.0001
Yes	72 (28.2)	17 (12.6)	55 (45.8)	

No	182 (71.4)	117 (86.7)	65 (54.2)	
Don't Know	1 (0.4)	1 (0.7)	0 (0.0)	
Gravidity				< 0.0001
None	91 (35.7)	64 (47.4)	27 (22.5)	
1	58 (22.7)	28 (20.7)	30 (25.0)	
2	47 (18.4)	25 (18.5)	22 (18.3)	
3 +	59 (23.1)	18 (13.3)	41 (34.2)	
Community Type				0.004
L. crispatus-dominant	52 (20.4)	37 (27.4)	15 (12.5)	
L. gasseri-dominant	3 (1.2)	1 (0.7)	2 (1.7)	
L. iners-dominant	99 (38.8)	43 (31.9)	56 (46.7)	
Diverse	98 (38.4)	51 (37.8)	47 (39.2)	
L. jensenii-dominant	3 (1.2)	3 (2.2)	0 (0.0)	
Candida				0.042
Positive	42 (16.5)	16 (11.9)	26 (21.7)	
Negative	213 (83.5)	119 (88.1)	94 (78.3)	

Values are n (%). Fisher's Exact Tests were used to determine p-values.

[a]Women who reported the oral contraceptive pill or the birth control ring;

[b]Women who reported the levonorgestrel-containing intrauterine device or depot medroxyprogesterone acetate;

[c]Women who reported condoms, rhythm/natural family planning, abstinence, withdrawal or nothing.

Supplemental Table 2: Vaginal *Candida* colonization by race and community type

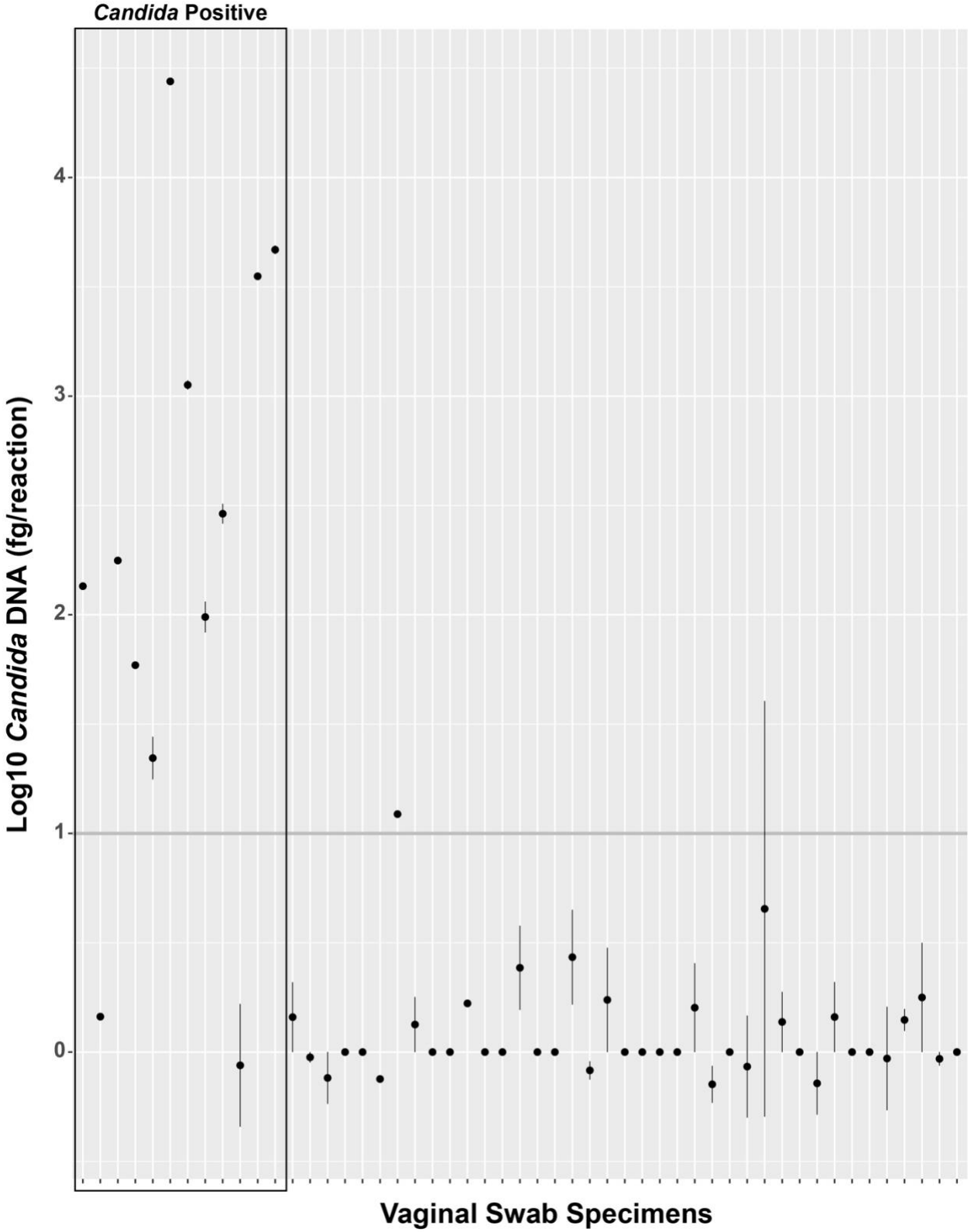
Characteristics	*Candida* Positive	*Candida* Negative
Total Number of Subjects	42 (16.5)	213 (83.5)
Black Race	26 (21.7)	94 (78.3)
L. crispatus-dominant	2 (13.3)	13 (86.7)
L. gasseri-dominant	0 (0.0)	2 (100.0)
L. iners-dominant	17 (30.4)	39 (69.6)
Diverse	7 (14.9)	40 (85.1)
L. jensenii-dominant	0 (0.0)	0 (0.0)
White Race	16 (11.9)	119 (88.1)
L. crispatus dominant	3 (8.1)	34 (91.9)
L. gasseri.-dominant	0 (0.0)	1 (100.0)
L. iners dominant	6 (14.0)	37 (86.0)
Diverse	6 (11.8)	45 (88.2)
L. jensenii dominant	1 (33.3)	2 (66.6)

Values are n (% of characteristic).

Supplemental Figure 1: Validation of *Candida* qPCR on 51 vaginal swab specimens

Fifty-one women were assessed for vaginal *Candida* colonization by culture and qPCR. The mean abundance and standard deviation of *Candida* DNA for each specimen are plotted. Culture positive specimens are indicated by the black box. Twelve specimens were culture positive for *Candida*, ten of which were also qPCR positive for *Candida.* The sensitivity of the qPCR diagnostic was 83.3% and the specificity was 100% for this set of samples.

Supplemental Figure 1



Sample ID	Nugent-defin	Candida Statu	Candida Spec	Normalized C	Race	BMI	Socioeconom	Community T	Curren Birth (	Age	Gravidity	Vaginal Douching in Last 180 D
YES_1	Intermediate	Negative		0	Black	Overweight	Low SES	L. iners-domi	Non-hormon	24	0	No
YES_2	Intermediate	Negative		0	White	Normal	Low SES	L. iners-domi	Estrogen + Pr	23	2	No
YES_3	Normal	Negative		0	Black	NA	Low SES	L. crispatus-d	Estrogen + Pr	24	0	Yes
YES_4	Normal	Negative		0	Black	Normal	Low SES	L. crispatus-d	Non-hormon	30	2	No
YES_5	BV	Negative		65.0969761	Black	Normal	Low SES	Diverse	Non-hormon	20	0	Yes
YES_6	Intermediate	Negative		0	Black	Obese	Low SES	L. iners-domi	Non-hormon	19	0	No
YES_7	BV	Positive	Candida albic	86570.0454	Black	Normal	Not Low SES	Diverse	Non-hormon	23	3	No
YES_8	Intermediate	Negative		0	White	Underweight	Not Low SES	L. crispatus-d	Non-hormon	21	3	No
YES_9	BV	Negative		1.44113294	White	Normal	Not Low SES	Diverse	Non-hormon	21	2	No
YES_10	Normal	Negative		0	White	Obese	Not Low SES	L. gasseri-dor	Non-hormon	32	2	No
YES_11	BV	Positive	Candida albic	8253.5563	Black	Normal	Not Low SES	Diverse	Estrogen + Pr	23	1	No
YES_12	BV	Negative		116.041788	White	Obese	Low SES	Diverse	Non-hormon	19	1	No
YES_13	Intermediate	Negative		0	White	Normal	Not Low SES	L. crispatus-d	Non-hormon	21	0	No
YES_14	Intermediate	Negative		0	Black	Obese	Low SES	L. iners-domi	Estrogen + Pr	22	1	No
YES_15	Normal	Negative		0	White	Normal	Low SES	L. crispatus-d	Non-hormon	20	0	No
YES_16	BV	Negative		6.10425286	Black	Obese	Low SES	Diverse	Non-hormon	18	0	No
YES_17	Intermediate	Negative		0	White	Obese	Low SES	L. iners-domi	Non-hormon	20	0	No
YES_18	BV	Negative		8.013427	Black	Overweight	Low SES	Diverse	Non-hormon	21	2	Yes
YES_19	BV	Negative		57.7992007	White	Underweight	Low SES	L. iners-domi	Non-hormon	22	1	No
YES_20	Normal	Negative		0	Black	Overweight	Low SES	L. crispatus-d	Non-hormon	24	1	No
YES_21	Intermediate	Positive	Candida glabı	1894.93576	White	Obese	Low SES	L. iners-domi	Non-hormon	34	3	No
YES_22	Normal	Negative		0	White	Obese	Low SES	L. iners-domi	Non-hormon	21	0	No
YES_23	Intermediate	Positive	Candida albic	187463.887	Black	Overweight	Not Low SES	L. iners-domi	Estrogen + Pr	26	1	No
YES_24	Normal	Positive	Candida albic	1481.17532	White	Normal	Not Low SES	L. iners-domi	Estrogen + Pr	23	0	No
YES_25	BV	Negative		58.2963389	White	Obese	Not Low SES	Diverse	Non-hormon	20	1	Yes
YES_26	Intermediate	Positive	Candida albic	208.050562	White	Normal	Not Low SES	Diverse	Non-hormon	21	0	No
YES_27	Intermediate	Negative		0	White	Normal	Not Low SES	L. iners-domi	Non-hormon	18	0	No
YES_28	Intermediate	Negative		0	Black	Normal	Low SES	L. iners-domi	Non-hormon	27	1	Yes
YES_29	Normal	Positive	Candida albic	28412.1258	Black	Overweight	Not Low SES	L. crispatus-d	Non-hormon	25	3	No
YES_30	Intermediate	Negative		0	Black	Overweight	Low SES	Diverse	Estrogen + Pr	20	1	Yes
YES_31	Normal	Negative		0	Black	Normal	Low SES	L. crispatus-d	Estrogen + Pr	24	1	No
YES_32	Normal	Negative		0	White	Normal	Not Low SES	L. crispatus-d	Non-hormon	23	0	No
YES_33	BV	Negative		0.25993252	Black	Obese	Low SES	Diverse	Non-hormon	23	3	Yes
YES_34	Normal	Negative		0	White	Normal	Not Low SES	L. crispatus-d	Estrogen + Pr	22	0	No
YES_35	BV	Negative		0	White	Normal	Not Low SES	Diverse	Estrogen + Pr	23	0	No
YES_36	BV	Negative		0	White	Normal	Low SES	Diverse	Non-hormon	29	3	Yes
YES_37	BV	Negative		0	White	Normal	Low SES	Diverse	Non-hormon	19	3	No
YES_38	BV	Positive	Candida glabı	29032.683	White	Normal	Not Low SES	Diverse	Estrogen + Pr	26	0	No

YES_41	BV	Negative		0	Black	NA	Not Low SES	Diverse	Non-hormona	20	1	No
YES_42	Intermediate	Negative		0	White	Normal	Not Low SES	L. iners-domi	Non-hormona	23	0	No
YES_43	Normal	Negative		0	White	Normal	Not Low SES	L. crispatus-d	Non-hormona	19	0	No
YES_44	Intermediate	Negative		8.1113445	White	Normal	Not Low SES	L. iners-domi	Non-hormona	25	0	No
YES_45	Intermediate	Negative		0	White	Obese	Not Low SES	Diverse	Estrogen + Pr	19	1	No
YES_46	Intermediate	Negative		0	Black	Obese	Not Low SES	L. iners-domi	Estrogen + Pr	18	0	Yes
YES_47	Normal	Negative		0	Black	Obese	Not Low SES	L. crispatus-d	Estrogen + Pr	40	1	Yes
YES_48	BV	Negative		0	Black	Obese	Low SES	Diverse	Estrogen + Pr	24	2	Yes
YES_49	Intermediate	Negative		0	White	Normal	Not Low SES	Diverse	Non-hormona	43	3	No
YES_50	BV	Positive	Candida albic	63.0981781	Black	Obese	Low SES	Diverse	Non-hormona	21	3	No
YES_51	BV	Positive	Candida albic	2438.76092	Black	Normal	Low SES	Diverse	Estrogen + Pr	21	3	No
YES_52	Normal	Negative		0	White	Overweight	Not Low SES	L. crispatus-d	Estrogen + Pr	23	0	No
YES_53	Normal	Negative		0	White	Normal	Not Low SES	L. crispatus-d	Estrogen + Pr	24	0	No
YES_54	Intermediate	Negative		13.7641916	Black	Obese	Low SES	L. iners-domi	Estrogen + Pr	22	3	Yes
YES_55	Intermediate	Negative		0	White	Normal	Low SES	L. iners-domi	Progestin	33	2	Yes
YES_56	Normal	Negative		0	White	Normal	Not Low SES	L. crispatus-d	Estrogen + Pr	20	0	No
YES_57	Normal	Negative		5.70184789	White	Obese	Low SES	L. crispatus-d	Non-hormona	22	1	No
YES_58	Intermediate	Negative		0	White	Overweight	Not Low SES	L. iners-domi	Non-hormona	33	2	No
YES_59	Intermediate	Negative		0	White	Overweight	Low SES	L. iners-domi	Non-hormona	19	1	No
YES_60	Intermediate	Negative		0	White	Overweight	Not Low SES	L. iners-domi	Non-hormona	38	3	No
YES_61	Normal	Negative		0	White	Normal	Not Low SES	L. iners-domi	Non-hormona	33	1	No
YES_62	Intermediate	Negative		0	White	Underweight	Not Low SES	L. iners-domi	Non-hormona	21	0	No
YES_63	Normal	Negative		0	Black	Obese	Low SES	L. iners-domi	Non-hormona	35	1	Yes
YES_64	Intermediate	Negative		8.2100207	Black	Obese	Low SES	L. iners-domi	Non-hormona	22	1	Yes
YES_65	Normal	Negative		0	White	Obese	Not Low SES	L. iners-domi	Progestin	37	3	No
YES_66	Intermediate	Negative		0	White	Normal	Low SES	L. iners-domi	Estrogen + Pr	22	0	No
YES_67	Normal	Negative		0	White	Obese	Not Low SES	L. iners-domi	Estrogen + Pr	20	1	No
YES_68	BV	Negative		0	Black	Normal	Low SES	Diverse	Non-hormona	22	1	No
YES_69	Intermediate	Negative		2.6648135	White	Normal	Not Low SES	Diverse	Non-hormona	25	2	No
YES_70	BV	Negative		0	White	Normal	Low SES	Diverse	Non-hormona	30	2	No
YES_71	Normal	Negative		0	White	Obese	Not Low SES	L. crispatus-d	Non-hormona	20	0	No
YES_72	Normal	Negative		2.2703877	White	Normal	Not Low SES	L. crispatus-d	Estrogen + Pr	25	0	No
YES_73	Normal	Negative		0	White	Normal	Not Low SES	L. iners-domi	Estrogen + Pr	24	2	Yes
YES_74	Normal	Negative		0	White	Normal	Low SES	L. iners-domi	Estrogen + Pr	28	1	No
YES_75	BV	Negative		2.96112474	White	Normal	Low SES	Diverse	Non-hormona	28	3	Yes
YES_76	Normal	Negative		0	White	Normal	Not Low SES	Diverse	Estrogen + Pr	30	2	No
YES_77	Intermediate	Negative		0	Black	Underweight	Low SES	Diverse	Non-hormona	24	3	Yes
YES_78	Intermediate	Negative		0	White	Normal	Not Low SES	L. iners-domi	Non-hormona	23	1	No
YES_79	Intermediate	Negative		0	Black	Overweight	Not Low SES	Diverse	Non-hormona	23	2	No

YES_82	BV	Negative		0	White	Normal	Low SES	Diverse	Non-hormona	24	0	No
YES_83	BV	Negative		2.65649706	White	Normal	Not Low SES	Diverse	Non-hormona	22	1	Yes
YES_84	Normal	Negative		0.79815645	White	Normal	Low SES	L. crispatus-d	Non-hormona	26	2	No
YES_85	Normal	Positive	Candida albic	328.284827	White	Normal	Not Low SES	L. crispatus-d	Estrogen + Pr	24	0	No
YES_86	Intermediate	Negative		0	Black	NA	Not Low SES	Diverse	Non-hormona	21	0	No
YES_87	Normal	Negative		0	White	Normal	Not Low SES	L. iners-domi	Non-hormona	18	1	No
YES_88	Intermediate	Negative		0	White	Obese	Low SES	L. iners-domi	Non-hormona	23	0	No
YES_89	BV	Positive	Candida albic	7023.12315	White	Overweight	Not Low SES	Diverse	Non-hormona	23	0	No
YES_90	Normal	Negative		23451.8114	White	Obese	Not Low SES	L. iners-domi	Estrogen + Pr	24	0	No
YES_91	Intermediate	Negative		0	White	Normal	Not Low SES	L. iners-domi	Non-hormona	19	0	No
YES_92	Normal	Negative		0	White	Normal	Low SES	L. crispatus-d	Non-hormona	25	3	Yes
YES_93	Intermediate	Negative		2.70333166	White	Normal	Low SES	Diverse	Estrogen + Pr	21	1	Yes
YES_94	Intermediate	Negative		0	White	Normal	Low SES	Diverse	Non-hormona	38	3	No
YES_95	Normal	Negative		0.81906327	Black	Obese	Not Low SES	L. iners-domi	Estrogen + Pr	19	0	No
YES_96	Normal	Negative		0	White	Obese	Not Low SES	L. crispatus-d	Estrogen + Pr	31	1	Yes
YES_97	Intermediate	Negative		0	Black	Overweight	Not Low SES	Diverse	Estrogen + Pr	31	3	No
YES_98	Intermediate	Negative		0	Black	Normal	Low SES	L. iners-domi	Non-hormona	25	1	No
YES_99	BV	Negative		0	White	Obese	Low SES	Diverse	Non-hormona	21	0	No
YES_100	Intermediate	Negative		1.79653156	Black	NA	Low SES	L. iners-domi	Non-hormona	23	3	Yes
YES_101	Normal	Negative		10.5067974	White	Normal	Low SES	L. iners-domi	Non-hormona	21	0	No
YES_102	Normal	Negative		0	White	Overweight	Not Low SES	L. crispatus-d	Estrogen + Pr	25	1	No
YES_103	Normal	Positive	Candida albic	12340.7547	White	Normal	Not Low SES	L. crispatus-d	Non-hormona	21	0	No
YES_104	Intermediate	Positive	Candida albic	24436.127	Black	Obese	Low SES	L. iners-domi	Non-hormona	29	3	Yes
YES_105	BV	Positive	Candida albic	1499.42015	Black	Underweight	Low SES	L. iners-domi	Non-hormona	17	1	No
YES_106	Normal	Negative		42.2505624	White	Normal	Not Low SES	L. crispatus-d	Non-hormona	24	0	No
YES_107	Intermediate	Positive	Candida albic	38623.9263	Black	Overweight	Low SES	L. iners-domi	Estrogen + Pr	25	3	No
YES_108	Intermediate	Negative		0	Black	Obese	Low SES	L. iners-domi	Non-hormona	24	0	No
YES_109	BV	Negative		162.516486	Black	Obese	Low SES	Diverse	Non-hormona	21	1	No
YES_110	Intermediate	Positive	Candida albic	9540.73006	Black	Overweight	Low SES	L. iners-domi	Non-hormona	36	3	No
YES_111	Normal	Negative		0	White	Overweight	Not Low SES	L. crispatus-d	Non-hormona	25	0	No
YES_112	Normal	Negative		0	Black	Obese	Low SES	L. iners-domi	Non-hormona	34	3	No
YES_113	Normal	Negative		0	White	Normal	Not Low SES	L. crispatus-d	Non-hormona	24	0	No
YES_114	BV	Negative		0	Black	Obese	Low SES	Diverse	Estrogen + Pr	20	0	Yes
YES_115	Intermediate	Negative		0	White	Normal	Not Low SES	Diverse	Non-hormona	24	0	No
YES_116	Intermediate	Negative		9.02890426	White	NA	Low SES	Diverse	Non-hormona	28	3	Yes
YES_117	Intermediate	Positive	Candida albic	42.1348947	Black	Underweight	Not Low SES	L. iners-domi	Non-hormona	19	2	Yes
YES_118	BV	Negative		0	White	Normal	Low SES	Diverse	Non-hormona	37	2	No
YES_119	Intermediate	Negative		1.28036551	White	Overweight	Not Low SES	Diverse	Progestin	17	0	No
YES_120	Intermediate	Negative		0	Black	Normal	Low SES	L. iners-domi	Progestin	22	1	No

YES_123	BV	Negative		0	Black	Overweight	Low SES	Diverse	Non-hormona	29	3 Yes
YES_124	Intermediate	Negative		2.71624999	White	Overweight	Not Low SES	Diverse	Non-hormona	24	1 No
YES_125	Normal	Negative		0	Black	Normal	Not Low SES	L. crispatus-d	Non-hormona	19	0 No
YES_126	Intermediate	Negative		26.4265269	Black	Normal	Not Low SES	Diverse	Non-hormona	24	0 Yes
YES_127	Intermediate	Negative		0	Black	Obese	Low SES	Diverse	Non-hormona	20	2 No
YES_128	Intermediate	Negative		0	White	Obese	Not Low SES	L. crispatus-d	Non-hormona	24	0 No
YES_129	Intermediate	Negative		0.39771533	Black	Overweight	Not Low SES	L. crispatus-d	Estrogen + Pr	19	0 No
YES_130	Intermediate	Negative		0	White	Normal	Not Low SES	L. crispatus-d	Non-hormona	25	1 No
YES_131	Intermediate	Negative		0	White	Obese	Low SES	Diverse	Non-hormona	34	2 No
YES_132	Intermediate	Negative		0	White	Normal	Not Low SES	Diverse	Non-hormona	20	1 No
YES_133	Normal	Negative		0	Black	Normal	Low SES	L. iners-domi	Non-hormona	26	3 Yes
YES_134	Intermediate	Negative		0	White	Overweight	Not Low SES	Diverse	Estrogen + Pr	21	1 No
YES_135	Normal	Positive	Candida albic	4134.34169	White	Normal	Not Low SES	L. crispatus-d	Estrogen + Pr	21	0 No
YES_136	Normal	Negative		0	White	Overweight	Not Low SES	L. iners-domi	Estrogen + Pr	27	2 No
YES_137	Intermediate	Negative		0	Black	Obese	Low SES	L. iners-domi	Non-hormona	24	3 No
YES_138	Normal	Negative		0	Black	Obese	Not Low SES	L. iners-domi	Estrogen + Pr	39	3 No
YES_139	Normal	Negative		11.8700646	Black	Obese	Not Low SES	L. crispatus-d	Non-hormona	26	3 Yes
YES_140	Normal	Negative		0	Black	Normal	Low SES	L. crispatus-d	Non-hormona	19	0 Yes
YES_141	Normal	Negative		0	Black	Obese	Not Low SES	L. gasseri-dor	Non-hormona	28	3 No
YES_142	Normal	Negative		0	Black	Obese	Low SES	L. iners-domi	Non-hormona	27	3 Yes
YES_143	Normal	Negative		0	Black	Underweight	Not Low SES	L. iners-domi	Estrogen + Pr	29	1 No
YES_144	Normal	Negative		0	Black	Normal	Low SES	L. iners-domi	Estrogen + Pr	21	2 No
YES_145	Intermediate	Negative		0.25398265	Black	Overweight	Low SES	Diverse	Non-hormona	22	3 Yes
YES_146	Normal	Negative		0	White	Underweight	Low SES	L. crispatus-d	Non-hormona	22	0 No
YES_147	Intermediate	Negative		0	Black	Normal	Not Low SES	Diverse	Estrogen + Pr	20	1 No
YES_148	Intermediate	Negative		0	White	Overweight	Low SES	Diverse	Non-hormona	20	1 No
YES_149	Normal	Negative		0	White	Normal	Low SES	L. iners-domi	Non-hormona	26	2 No
YES_150	Normal	Negative		23.4111701	Black	Overweight	Low SES	L. crispatus-d	Non-hormona	29	2 No
YES_151	Intermediate	Negative		69.6541605	White	Obese	Not Low SES	L. crispatus-d	Non-hormona	25	1 No
YES_152	Normal	Positive	Candida albic	568.299559	Black	Normal	Low SES	L. iners-domi	Estrogen + Pr	26	0 No
YES_153	BV	Negative		1.61002131	Black	Normal	Low SES	Diverse	Progestin	18	0 Yes
YES_154	Intermediate	Negative		0	White	Normal	Not Low SES	L. jensenii-do	Non-hormona	18	0 No
YES_155	Intermediate	Negative		0	White	Underweight	Low SES	L. iners-domi	Estrogen + Pr	20	2 No
YES_156	Normal	Negative		0	Black	Normal	Not Low SES	L. iners-domi	Non-hormona	23	0 No
YES_157	Normal	Negative		2.1838732	White	Normal	Not Low SES	L. crispatus-d	Estrogen + Pr	26	0 No
YES_158	Normal	Negative		0	White	Overweight	Not Low SES	L. crispatus-d	Estrogen + Pr	22	0 No
YES_159	Intermediate	Negative		0	White	Normal	Not Low SES	Diverse	Non-hormona	20	1 Yes
YES_160	Intermediate	Negative		12.133129	White	Obese	Low SES	Diverse	Non-hormona	20	0 No
YES_161	Normal	Negative		0	Black	Obese	Low SES	L. iners-domi	Non-hormona	20	2 No

YES_164	Normal	Negative		0	White	Normal	Not Low SES	L. iners-domi	Non-hormon	21	0	No
YES_165	Intermediate	Positive	Candida albic	47.6500989	White	Obese	Not Low SES	L. iners-domi	Estrogen + Pr	23	0	No
YES_166	Intermediate	Negative		4.99994678	Black	Overweight	Low SES	L. iners-domi	Non-hormon	21	2	No
YES_167	Normal	Negative		0	White	Normal	Low SES	L. iners-domi	Non-hormon	20	0	No
YES_168	Normal	Negative		0	White	Obese	Low SES	L. crispatus-d	Non-hormon	44	2	No
YES_169	Normal	Negative		0	White	Overweight	Not Low SES	L. iners-domi	Estrogen + Pr	20	0	No
YES_170	Normal	Negative		0	White	Normal	Not Low SES	L. iners-domi	Estrogen + Pr	23	1	No
YES_171	Normal	Negative		0	Black	Normal	Low SES	Diverse	Non-hormon	26	3	Yes
YES_172	Normal	Negative		1.15549064	White	Underweight	Not Low SES	L. crispatus-d	Non-hormon	22	0	No
YES_173	Normal	Negative		0	White	Underweight	Not Low SES	L. crispatus-d	Non-hormon	25	0	No
YES_174	Normal	Negative		0	White	Normal	Not Low SES	L. crispatus-d	Estrogen + Pr	23	0	No
YES_175	Intermediate	Positive	Candida albic	713.767451	White	Normal	Low SES	Diverse	Estrogen + Pr	23	2	No
YES_176	Intermediate	Negative		0	White	Normal	Not Low SES	Diverse	Estrogen + Pr	21	1	No
YES_177	Intermediate	Negative		0	White	Obese	Low SES	L. iners-domi	Non-hormon	26	2	No
YES_178	Intermediate	Negative		0	White	Obese	Not Low SES	L. iners-domi	Non-hormon	24	0	No
YES_179	Intermediate	Positive	Candida albic	563.647457	Black	Normal	Low SES	L. iners-domi	Non-hormon	19	0	No
YES_180	Intermediate	Positive	Candida glabı	3342.61464	White	Normal	Not Low SES	Diverse	Non-hormon	25	3	Yes
YES_181	Intermediate	Negative		0.95358647	White	Normal	Low SES	Diverse	Non-hormon	26	2	Yes
YES_182	Intermediate	Negative		0	Black	Obese	Low SES	Diverse	Non-hormon	26	1	Yes
YES_183	Normal	Negative		0	White	Normal	Not Low SES	L. jensenii-do	Non-hormon	22	0	Yes
YES_184	Normal	Negative		0	White	Overweight	Low SES	L. crispatus-d	Non-hormon	27	2	No
YES_185	BV	Negative		6.4605685	White	Obese	Not Low SES	Diverse	Estrogen + Pr	24	1	No
YES_186	BV	Negative		1.5078887	White	Overweight	Low SES	Diverse	Non-hormon	18	1	No
YES_187	Intermediate	Negative		0	Black	Overweight	Low SES	Diverse	Progestin	38	3	No
YES_188	Intermediate	Negative		0	White	NA	Not Low SES	Diverse	Non-hormon	25	0	No
YES_189	Intermediate	Negative		0	White	Normal	Not Low SES	Diverse	Estrogen + Pr	37	3	Unknown
YES_190	Intermediate	Negative		0.57756761	Black	Normal	Not Low SES	L. iners-domi	Non-hormon	17	0	No
YES_191	Intermediate	Negative		0	Black	Obese	Not Low SES	Diverse	Non-hormon	24	1	Yes
YES_192	Intermediate	Negative		14.506964	Black	Obese	Not Low SES	Diverse	Non-hormon	32	2	Yes
YES_193	Normal	Positive	Candida albic	173082.034	Black	Obese	Low SES	L. crispatus-d	Estrogen + Pr	29	1	Yes
YES_194	Intermediate	Negative		6.52451099	Black	Normal	Not Low SES	L. gasseri-dor	Non-hormon	31	1	No
YES_195	Normal	Positive	Candida albic	1099.04235	Black	Normal	Low SES	L. iners-domi	Estrogen + Pr	32	3	No
YES_196	Intermediate	Negative		1.10024304	White	Normal	Not Low SES	Diverse	Non-hormon	39	2	No
YES_197	Intermediate	Negative		5.52333002	White	Normal	Low SES	Diverse	Non-hormon	26	0	Yes
YES_198	BV	Negative		12.3443957	White	NA	Low SES	Diverse	Non-hormon	26	2	No
YES_199	Intermediate	Negative		0	Black	Overweight	Not Low SES	Diverse	Estrogen + Pr	31	2	Yes
YES_200	Intermediate	Negative		0	White	Overweight	Not Low SES	Diverse	Non-hormon	22	0	No
YES_201	Intermediate	Positive	Candida albic	3775.30361	Black	Normal	Low SES	L. iners-domi	Non-hormon	23	1	No
YES_202	Normal	Negative		4.56336091	White	Overweight	Low SES	L. crispatus-d	Estrogen + Pr	23	0	No

YES_205	Normal	Positive	Candida albic	42294.0128	White	NA	Not Low SES	L. iners-domi	Non-hormon	23	0	No
YES_206	Normal	Positive	Candida albic	62174.0822	Black	Overweight	Low SES	L. iners-domi	Non-hormon	31	2	No
YES_207	Intermediate	Negative		1.40141557	Black	Obese	Not Low SES	L. iners-domi	Non-hormon	20	0	Yes
YES_208	Normal	Negative		0	White	Normal	Not Low SES	L. iners-domi	Estrogen + Pr	23	0	No
YES_209	Normal	Negative		6.47304481	White	NA	Low SES	Diverse	Non-hormon	31	3	No
YES_210	Intermediate	Positive	Candida albic	806.516955	White	Overweight	Low SES	L. jensenii-do	Non-hormon	21	0	No
YES_211	Normal	Negative		2.62901394	White	Normal	Low SES	L. crispatus-d	Non-hormon	34	3	No
YES_212	Normal	Negative		1.18513934	Black	Overweight	Not Low SES	L. iners-domi	Progestin	30	1	No
YES_213	Normal	Positive	Candida albic	706.047377	White	Normal	Low SES	L. iners-domi	Non-hormon	23	2	No
YES_214	BV	Negative		0	Black	Obese	Low SES	Diverse	Non-hormon	32	3	Yes
YES_215	Normal	Negative		0	White	Normal	Low SES	L. iners-domi	Non-hormon	21	3	No
YES_216	BV	Negative		0	Black	Obese	Low SES	Diverse	Non-hormon	33	2	Yes
YES_217	Normal	Negative		1.70115654	Black	Normal	Not Low SES	L. iners-domi	Estrogen + Pr	31	3	No
YES_218	Intermediate	Positive	Candida albic	143133.167	White	Underweight	Not Low SES	L. iners-domi	Non-hormon	19	0	No
YES_219	Normal	Negative		0	White	Overweight	Low SES	L. crispatus-d	Estrogen + Pr	34	3	No
YES_220	BV	Negative		0	Black	Normal	Low SES	Diverse	Non-hormon	32	3	No
YES_221	Intermediate	Positive	Candida albic	707.844725	White	Overweight	Not Low SES	Diverse	Estrogen + Pr	22	0	No
YES_222	Normal	Negative		0	Black	Obese	Low SES	L. crispatus-d	Progestin	26	1	Yes
YES_223	Intermediate	Negative		0	Black	Overweight	Not Low SES	Diverse	Non-hormon	20	1	Yes
YES_224	Intermediate	Negative		0	Black	Overweight	Low SES	L. iners-domi	Estrogen + Pr	27	3	No
YES_225	BV	Negative		0	White	Obese	Not Low SES	Diverse	Non-hormon	21	0	Yes
YES_226	Normal	Positive	Candida glabı	726.484911	Black	NA	Low SES	L. iners-domi	Progestin	18	2	No
YES_227	Normal	Negative		0	Black	Overweight	Low SES	L. crispatus-d	Non-hormon	28	3	Yes
YES_228	Intermediate	Negative		3.87273945	Black	Normal	Low SES	Diverse	Non-hormon	23	2	No
YES_229	Intermediate	Positive	Candida albic	447096.875	Black	Overweight	Not Low SES	Diverse	Non-hormon	22	3	No
YES_230	Normal	Negative		0	White	Obese	Low SES	Diverse	Non-hormon	22	1	No
YES_231	Normal	Negative		0	Black	Obese	Low SES	L. iners-domi	Progestin	32	2	Yes
YES_232	BV	Negative		0	Black	Obese	Not Low SES	Diverse	Non-hormon	28	0	Yes
YES_233	Normal	Negative		0	White	Underweight	Low SES	L. crispatus-d	Non-hormon	24	1	No
YES_234	Normal	Negative		0	Black	Underweight	Low SES	L. iners-domi	Non-hormon	25	3	Yes
YES_235	Normal	Positive	Candida albic	7528.63518	Black	Overweight	Low SES	L. iners-domi	Estrogen + Pr	29	1	Yes
YES_236	Intermediate	Negative		5.86670172	Black	Obese	Low SES	Diverse	Non-hormon	29	2	No
YES_237	Intermediate	Negative		3.62933363	Black	Normal	Low SES	Diverse	Non-hormon	21	1	No
YES_238	Intermediate	Negative		0	Black	Obese	Low SES	L. iners-domi	Non-hormon	23	0	Yes
YES_239	Intermediate	Positive	Candida albic	3332.48181	Black	Normal	Low SES	Diverse	Estrogen + Pr	36	3	Yes
YES_240	Intermediate	Negative		0	Black	Obese	Low SES	Diverse	Progestin	44	2	Yes
YES_241	Intermediate	Negative		0.84273838	Black	Obese	Low SES	Diverse	Non-hormon	20	0	Yes
YES_242	Intermediate	Negative		0.44740179	Black	Obese	Low SES	L. iners-domi	Non-hormon	22	3	Yes
YES_243	Intermediate	Positive	Candida albic	512142.994	Black	Overweight	Low SES	L. iners-domi	Non-hormon	32	3	No

YES_246	Intermediate	Negative		2.46120436	Black	Obese	Low SES	Diverse	Non-hormona	17	0	No
YES_247	Normal	Positive	Candida albic	1048.67416	Black	Normal	Low SES	L. iners-domi	Non-hormona	31	3	Yes
YES_248	BV	Negative		0.51121459	White	Obese	Low SES	Diverse	Estrogen + Pr	32	0	No
YES_249	BV	Negative		0	White	Obese	Not Low SES	Diverse	Non-hormona	25	0	No
YES_250	Normal	Negative		0	Black	Obese	Low SES	L. iners-domi	Estrogen + Pr	41	3	Yes
YES_251	Normal	Negative		5.25077639	Black	Normal	Low SES	L. iners-domi	Estrogen + Pr	26	0	Yes
YES_252	Normal	Negative		0.66421679	Black	Obese	Low SES	L. iners-domi	Non-hormona	20	0	Yes
YES_253	Normal	Negative		0	Black	Underweight	Not Low SES	L. crispatus-d	Non-hormona	19	2	Yes
YES_254	Normal	Negative		181.228631	Black	Obese	Low SES	L. iners-domi	Non-hormona	37	3	No
YES_255	Normal	Negative		5.38271329	Black	Obese	Low SES	L. iners-domi	Non-hormona	21	1	Yes